abcam

Product datasheet

HRP Conjugation Kit - Lightning-Link® ab102890

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概述

产品名称

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HRP**偶**联试剂**盒** - Lightning-Link®

HRP Conjugation Kit / HRP Labeling Kit ab102890 uses a simple and quick process for HRP labeling / conjugation of antibodies. It can also be used to conjugate other proteins or peptides. Learn about our **antibody labeling kits and their advantages**.

To conjugate an antibody to HRP using this kit:

- add modifier to antibody and incubate for 3 hrs
- add quencher and incubate for 30 mins

The HRP conjugated antibody can be used immediately in WB, ELISA, IHC etc. No further purification is required and 100% of the antibody is recovered for use.

Learn about buffer compatibility below; for incompatible buffers and low antibody concentrations, use our rapid <u>antibody purification and concentration kits</u>. Use the <u>FAQ</u> to learn more about the technology, or about conjugating other proteins and peptides to HRP.

Custom size conjugation kits up to 100 mg are available on demand. Please contact us to discuss your requirements.

This product is manufactured by Expedeon, an Abcam company, and was previously called Lightning-Link[®] HRP Labeling Kit. 701-0004 is the same as the 5 mg size. 701-0003 is the same as the 5 x 1 mg size. 701-0000 is the same as the 3 x 100 ug size. 701-0030 is the same as the 3 x 10 ug size. 701-0010 is the same as the 1 mg size.

Amount and volume of antibody for conjugation to HRP

Kit size	Recommended amount of antibody ¹	Maximum amount of antibody	Maximum antibody volume ²	
3 x 10 µg	3 x 10 µg	3 x 40 µg	3 x 10 µL	
100 μg	1 x 100 μg	1 x 400 μg	1 x 100 μL	
3 x 100 µg	3 x 100 μg	3 x 400 μg	3 x 100 μL	
1 mg	1 x 1 mg	1 x 4 mg	1 x 1 mL	

说明

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5 x 1 mg	5 x 1 mg	5 x 4 mg	5 x 1 mL
5 mg	1 x 5 mg	1 x 20 mg	1 x 5 mL

¹ Recommended amount of antibody will give an HRP:antibody molar ratio of 4:1. Antibodies often also perform well at the maximum amount of antibody which is 1:1 molar ratio.

Buffer Requirements for Conjugation

Buffer should be pH 6.5-8.5.

Buffer constituent compatibility

If a concentration is shown, then the constituent should be no more than the concentration shown. If several constituents are close to the limit of acceptable concentration, then this can inhibit conjugation.

Compatible buffer constituents			
50mM / 0.6% Tris ¹	0.1% BSA	50% glycerol	
0.1% sodium azide ²	PBS	Potassium phosphate	
Sodium chloride	HEPES	Sucrose	
Sodium citrate	EDTA	Trehalose	

¹ Tris buffered saline is almost always ≤ 50 mM / 0.6%

² Sodium azide irreversibly inhibits HRP; antibodies with azide should be purified before using the HRP conjugation kit.

Incompatible buffer constituents			
Thiomerosal	Proclin	Glycine	
Arginine	Glutathione	DTT	

If a constituent of the buffer containing your antibody or protein is not listed above, please check the **FAQ** or **contact us**.

Only purified antibodies are suitable for use, ie. where other proteins, peptides, or amino acids are not present: antibodies in ascites fluid, serum or hybridoma culture media are incompatible.

Storing and handling conjugation kits

Lyophilized Lightning-Link $^{\circledR}$ components are hygroscopic.

Kits are intentionally shipped at ambient temperature with silica gel to avoid exposure to moisture. Upon receipt, store the kit frozen and protect from moisture. Before opening the outer container, allow the lyophilized components to reach room temperature to minimize condensation.

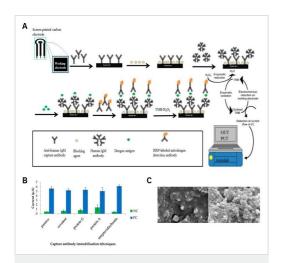
 $^{^2}$ Ideal antibody concentration is 1mg/ml. 0.5 - 1 mg/ml can be used if the maximum antibody volume is not exceeded. Antibodies > 4 mg/ml or < 0.5 mg/ml should be diluted /concentrated.

存放说明

Store at -20°C. Please refer to protocols.

组 件	100 µg	1 mg	3 x 10 μg	5 x 1 mg	3 x 100 µg	1 x 5 mg
HRP mix	1 x 100µg	1 x 1mg	3 x 10μg	5 x 1mg	3 x 100µg	1 x 5mg
Modifier reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 1200µl	1 x 200µl	1 x 1200µl
Quencher reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 1200µl	1 x 200µl	1 x 1200µl

图片

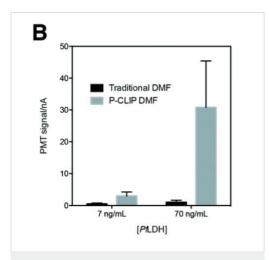


Conjugation - HRP Conjugation Kit - Lightning-Link® (ab102890)

Image from Parkash, Om, et al., Diagnostics (Basel), 11(1):33; doi: 10.3390/diagnostics11010033. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Parkash, Om, et al used HRP Conjugation Kit - Lightning-Link[®] (ab102890) as part of the development and evaluation of a biosensor based on screen-printed carbon electrodes (SPCEs) for the detection of dengue-specific immunoglobulin M (lgM) antibodies. They used the kit to conjugate HRP to anti-dengue antibody for use in conjugation.

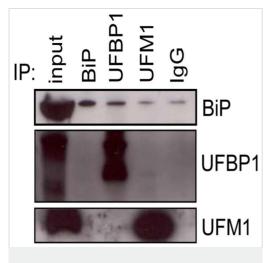
(A) Schematic diagram of the screen printed carbon electrode (SCPE)-based dengue IgM biosensor. The biosensor was constructed by sequentially adding optimised concentration of antihuman IgM capture antibody, blocking agent, human IgM antibody or serum sample, dengue antigen and detection antibody, with washing steps in between. Electrochemical signal was generated following addition of TMB substrate. (B) Comparison of various immobilisation techniques for the goat anti-human IgM capture antibody. NC: negative control consisting of a dengue IgM negative serum sample; PC: dengue IgM positive serum sample. (C) Field Emission Scanning Electron Microscopy (FESEM) surface images of (left panel) a bare carbon electrode and (right panel) a carbon electrode modified with an anti-human IgM antibody using the streptavidin/biotin immobilisation system. Both capture and detection antibodies were labeled using Lightning-Link® conjugation kits (ab201796 and ab102890).



Conjugation - HRP Conjugation Kit - Lightning-Link® (ab102890)

Image from Rackus et al., Lab Chip., 17(13):2272-2280; doi: 10.1039/c7lc00440k.Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/3.0/

Rackus, Darius G., et al used HRP Conjugation Kit - Lightning-Link (ab102890) as part of examining digital microfluidics for detection a malaria biomarker. They used the kit to conjugate HRP to anti-Plasmodium falciparum LDH antibody for use in preconcentration by liquid intake by paper (P-CLIP). DMF immunoassay for PfLDH. Comparison of mean signals obtained by traditional DMF-ELISA (black) and P-CLIP modified DMF-ELISA (grey) for concentrations below the limit of detection (7 ng mL $^{-1}$) and limit of quantitation (70 ng mL $^{-1}$) of the traditional DMF-ELISA. Error bars \pm 1 std. dev. (n = 3).

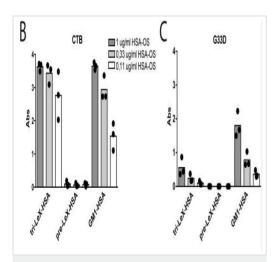


Western blot - HRP Conjugation Kit- Lightning-Link(ab102890)

Image from Lemaire, Katleen, et al., PloS one, 6(4): e18517; doi: 10.1371/journal.pone.0018517. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Lemaire, Katleen, et al used HRP Conjugation Kit - Lightning-Link[®] (ab102890) as part of examining protein interactions. They used the kit to conjugate HRP to anti-BIP and anti-UFBP1 antibodies for use in detection by western blot after immunoprecipitation.

Co-immunoprecipitation with BiP, UFL1 and UFM1 specific antibodies. BiP and UFBP1 antibodies were conjugated to HRP using ab102890 for detection after immunoprecipitation.

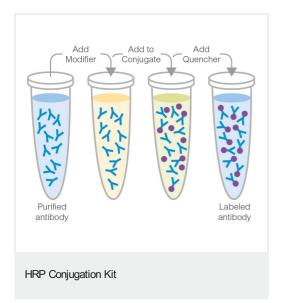


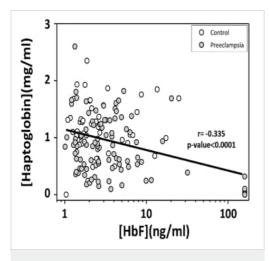
ELISA - HRP Conjugation Kit Lightning-Link

Image from Cervin, Jakob, et al., PLoS pathogens?14.2 (2018): e1006862. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Cervin, Jakob, et al used HRP Conjugation Kit - Lightning-Link[®] (ab102890) as part of examining binding of Cholera Toxin subunit B (CTB) to blood group antigen LewisX (LeX). They used the kit to conjugate HRP to CTB and G33D (mutated version of CTB) for use in ELISA.

ELISA with titrated amounts human serum albumin-linked oligosaccharides (HSA-OS), immobilized to wells and detected with (B) CTB-HRP and (C) G33D-HRP. Graph shows absorbance values from three independent experiments.



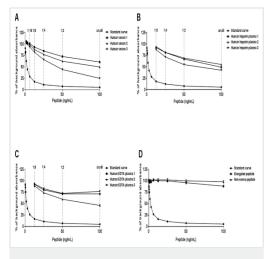


HRP Conjugation Kit - Lightning-Link® labeling monoclonal and polyclonal rabbit IgG for ELISA

Image from Gram Met al.,PLoS One, 10(9):e0138111. Fig 1.; doi: 10.1371/journal.pone.0138111. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Gram M et al. used ab102890 to assess levels of cell free fetal haemoglobin (HbF) and haptoglobin (Hp) by ELISA.

Samples were from normal pregnancies (Control) and women diagnosed with PE. The cell-free HbF plasma concentration of each patient sample (Control and PE) was plotted against the Hp plasma concentration.

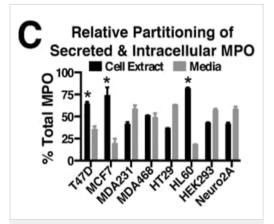


HRP Conjugation Kit - Lightning-Link® labeling C3C antibody for competitive ELISA

Image from Rasmussen DG et al., PLoS One, 12(1):e0170023. Fig 2.; doi: 10.1371/journal.pone.0170023. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Rasmussen DG et al. used ab102890 to determine levels of C3C by competitive ELISA.

(A) Standard curve and inhibition of the competitive ELISA signal using healthy human serum, (B) human heparin plasma and (C) human EDTA plasma. The native material was run from undiluted and up to 8-fold diluted as indicated. (D) Neo-epitope specificity of the C3C ELISA was shown by comparing reactivity towards an elongated peptide, i.e. a peptide with an additional amino acid at the N-terminal generated by cleavage, a non-sense peptide, i.e. a peptide with a different sequence, and the standard peptide. The standard peptide (i.e. standard curve), elongated peptide, and non-sense peptide were diluted 2-fold from 100 ng/mL. The data is presented as percent (%) of background absorbance, which is the absorbance with only assay buffer present, as a function of peptide concentration.

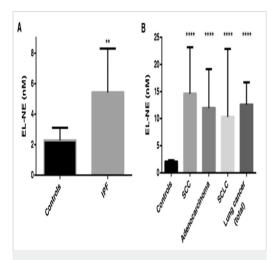


HRP Conjugation Kit - Lightning-Link® labeling polyclonal goat and Mab-16E3 anti-MPO antibodies for ELISA

Image from Laura RP et al., PloS One, 11(2):e0149391. Fig 2.; doi: 10.1371/journal.pone.0149391. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Laura RP et al. used ab102890 to determine concentrations of MPO by ELISA.

The relative amounts of secreted versus cellular myeloperoxidase (MPO) were calculated for each cell line.



HRP Conjugation Kit - Lightning-Link® labeling monoclonal EL-NE antibody for competitive ELISA

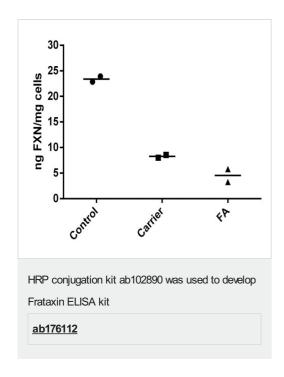
Image from Kristensen JH et al., BMC Pulm Med., 15:53. Fig 3.; doi: 10.1186/s12890-015-0048-5. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Kristensen JH et al. used ab102890 for the quantification of neutrophil elastase (NE)-degraded elastin (EL).

EL-NE fragment levels in serum from patients with IPF (n = 10) compared with controls (n = 9).

** p < 0.01. (B): EL-NE fragment levels in serum from patients diagnosed with lung cancer (n = 40 in total), SCC (n = 16), adenocarcinoma (n = 16) and SCLC (n = 8) compared with controls (n = 12). **** p < 0.0001. Groups were compared by T-test with Welch correction. Data are shown as the geometric mean (95% CI).

Abbreviations: IPF, idiopathic pulmonary fibrosis; SCC, squamous cell carcinoma; SCLC, small cell lung carcinoma.



HRP conjugation kit ab102890 is used by Abcam extensively in ELISA development. It is used for development / manufacturing of Abcam's SimpleStep ELISA® kits, including the Frataxin ELISA kit ab176112 used to produce the image above.

Transformed B lymphocyte cells from Friedreich's Ataxia (FA) samples were compared to heterozygous carrier B lymphocyte cells (Carrier) and control B lymphocyte cells (Control). B lymphocyte cell extracts were analyzed across a 7-point titration (0.1-100 µg/mL) and frataxin levels were interpolated from the standard curve. Average interpolated values of Frataxin are plotted.

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